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Effect of Acid and Metal Solutions on Seedling Foliage of Two Western Conifers

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ABSTRACT

As a precursor to field experiments now in progress to examine possible air pollution effects on Rocky Mountain conifer species, we conducted a greenhouse study to test the effects of a number of acid and metal solutions on foliage of lodgepole pine (*Pinus contorta* var. *latifolia*) and Douglas-fir (*Pseudotsuga menziesii*) seedlings. Seedlings were treated with a single immersion of foliage into three acids (HCL, H_2SO_4, and HNO_3), each at four different strengths (pH 5.0, 4.0, 3.0, and 2.0); and five metal chlorides ($ZnCl_2$, $CdCl_2$, $HgCl_2$, $CuCl_2$, and $PbCl_2$), each at four different molar (m) concentrations (10^{-4}, 10^{-3}, 10^{-2}, and 10^{-1} M). The control treatment was pH 6 distilled water. Injury to foliage was recorded after five weeks by counting dead needles and those having chlorotic bands or tips.

Statistically significant ($p < 0.05$) acid and metal effects were observed. Metal effects were greater than acid effects for both species. Significant acid injury occured only on Douglas-fir seedlings and only from H_2SO_4 and HNO_3 at pH 2; none of the acid treatments had significant effect on lodgepole pine. Foliage of both species was affected by all metals tested at concentrations of 10^{-2} M and stronger, with the exception of Pb which had statistically insignificant effects on lodgepole pine and affected Douglas-fir significantly only at 10^{-1} M.

INTRODUCTION

Injury to terrestrial ecosystems from airborne materials, including acids and metals, has been a concern for many years (Legge and Krupa, 1986). Simulated acid rain at pH 1-3 has been found to injure foliage of some conifers (Abouguendia and Baschak 1987, Haines et al 1980, and McColl and Johnson 1983). And highly acidic cloud water in areas of high pollutant emissions is blamed in part for declines in forest health -- the best example being that of high elevation red spruce (Picea rubens Sarg.) in the northern Appalachians (Hertel and McKinney-McNeal 1991). Although atmospheric acid is blamed for forest decline, air-soil effects and foliage-root effects have not been well-distinguished. While acid pollution has been relatively well studied in the populous Northeastern United States, the authors have found few reports on the effects of acids on Rocky Mountain conifers (Hertel and McKinney-McNeal 1991).

In the Rocky Mountains, metals may be more serious airborne pollutants than acids, because of smelters distributed throughout the region. While metals supplied to conifer roots have been found to have negative effects (Patterson and Olson 1983), we found no conifer studies of foliar absorption of metals. Metals were found to enter the free space within angiosperm leaves and cause leaf damage (Huges et al 1980, Chino 1981): thus, they are also likely to affect conifer leaves -- if they can gain entry.

We conducted a greenhouse experiment to gain information on the sensitivity of Rocky Mountain conifer seedlings to airborne acids and metals, and for designing a series of forest studies. Since information on direct effects of airborne acid and metal pollutants is inadequate, we tested experimentally applied acid and metal analogs for their effect on seedling foliage. Two of the most widespread conifers of the Rocky Mountains, lodgepole pine (Pinus contorta var. latifolia) and Douglas-fir (Pseudotsuga menziesii) were treated.

This paper reports extent of needle injury observed 5 weeks after the acid and metal treatments were applied, and discusses implications of long-term accumulative effects of airborne acids and, especially, airborne metals.

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METHODS

One-year-old lodgepole pine and Douglas-fir seedlings in plastic tubes (2.5cm diameter x 15 cm long) were obtained from the U.S. Forest Service and acclimatized for 90 days in a greenhouse under fluorescent lights with a light/dark cycle of 15/9 hours. Greenhouse temperatures were controlled within the range of 10 - 30 degrees C, and averaged 21 C during the day and 16 C at night. The seedlings were watered with tap water three times a week.

Treatment effects were evaluated with two factorial experiments, one for each species. Because the pine seedlings had a tendency toward chlorosis (yellowing) and a slight degree of needle mottling prior to treatments (whereas the Douglas-fir appeared uniformly dark green), the species were evaluated separately.

In each experiment, five seedlings of each species were randomly assigned to each acid X concentration treatment, including the distilled water control; and to each metal X concentration treatment, including the distilled water control. For the acid treatments, the above-ground portion of seedlings were dipped in solutions of three acids (HCL, H_2SO_4, and HNO_3) each with four pH's (5.0, 4.0, 3.0 and 2.0). The acid solutions were made by adding the appropriate amount of pure reagent to distilled water. Similarly, in metal treatments, the trees were dipped in solutions of $HgCl_2$, $CdCl_2$, $ZnCl_2$, $CuCl_2$, and $PbCl_2$ each at

molarities(M) of 10^{-4}, 10^{-3}, 10^{-2}, and 10^{1}. For each metal, a 1 molar metal chloride solution was made using distilled water and it was diluted serially to achieve the treatment molarities. Solution pH was adjusted to 6.0 using HCL or NaOH. Molarities of the solutions are presented as parts per million (ppm) in table 1 to facilitate comparison of our experiment with a literature often using ppm.

Seedlings were labelled with their replication and treatment codes prior to treatment. In order to treat the foliage without affecting the roots, each seedling was inverted and dipped in its treatment solution. First, however, the seedlings were dipped in a beaker of distilled water to remove any material adhering to the needles; any water adhering to the needles was shaken off. The seedling was then swirled in one of the treatment solutions for 5 seconds, and then swirled in a second solution of the same formulation for 5 seconds to minimize dilution effects of the initial distilled water rinse. The treated seedlings were placed in an inverted position to prevent the solution from running into the rooting medium while the foliage dried. When dry, the seedlings were returned to an upright position in trays and returned to the greenhouse for observation. To avoid contamination of the roots with treatment solutions while watering the seedlings, they were watered by immersing the tubes -- a tray at a time -- in a tap water bath until the soil was saturated, but not allowing water to contact stems or needles. Needle injury developed gradually following treatments

until, after 5 weeks, differences were visible among the treatments. At that time both the total number of needles and the number of injured needles -- those exhibiting chlorotic banding, tip chlorosis, or death -- were counted and recorded for each seedling. The number of injured needles (sum of tipped, banded,and dead needles) was expressed as a percentage of total needles for each seedling and used as the dependent variable in statistical analyses.

Data were analyzed by analysis of variance (ANOVA), using the General Linear Models procedures of the SAS statistical software (SAS Institute,Inc. 1987) to handle imbalance due to several missing values and to provide pairwise comparisons of appropriate contrasts. Each species was analyzed separately because of their aforementioned difference in initial needle appearance. Because raw data values of percent damage were not normally distributed, they were transformed with the ranking procedure of SAS and, after transformation, were found to be essentially normal in distribution. Ranked values of the dependent variable were thus used in all ANOVA's and were the basis of all statistical tests reported here; however, means of the raw data are also reported to indicate numerical differences among the various effects. The general analyses of variance indicated highly significant differences ($p < 0.01$) between acid treatments and metal treatments, for each species; and that treatment concentration was highly significant ($p < 0.01$) for metal treatments on both species, but only for acid treatments on Douglas-fir. To

determine which concentrations of which acids and metals were statistically different from the distilled water controls, probabilities of difference of least squares means estimates were computed with the SAS software. Pairwise comparison of concentrations within acids and metals was avoided, as was comparison of different concentrations of acids vs metals.

RESULTS

Acid Effects

Comparison of least squares means of ranked needle injury data, between acid-treated and control samples, showed significant acid effects only on Douglas-fir and only from H_2SO_4 and HNO_3, at pH2 (Table 4). None of the acid treatments had significant effect on lodgepole pine. In terms of raw data means (Table 2), the percentage of injured Douglas-fir needles from the pH2 concentration was 16 points higher for H_2SO_4 and 7 points higher for HNO_3 than their distilled water controls.

Metal Effects

Foliage of lodgepole pine and Douglas-fir seedlings was affected by all metal chlorides tested, at concentrations of 10^{-2} M and stronger, with the exception of $PbCl_2$ which had statistically insignificant effects on lodgepole pine and affected Douglas-fir only at 10^{-1} M (Table 5). $CdCl_2$ was also found to have

significant effects on Douglas-fir at the 10^{-3} M concentration. In relation to raw data means (Table 3), the average needle injury on lodgepole pine from injurious metal concentrations (10^{-1} and 10^{-2} M) showed an increase of 58 percentage points for Cd, 51 for Hg, 38 for Zn, and 32 for Cu, when compared to their controls. Needle injury on Douglas-fir from injurious metal concentrations showed an increase of 45 percentage points for Hg, 25 for Zn, 12 for Cu, 11 for Cd, and 8 for Pb -- in comparison to their distilled water controls. Although it is possible that a specific metal might affect Douglas-fir and lodgepole pine differently (e.g. Cu had a larger nominal effect on lodgepole pine than on Douglas-fir -- see Table 3), we could not make a valid statistical test of species differences.

DISCUSSION

Acid Effects

Since the acidity of rainfall is usually weaker than pH 3.0 (Knapp et al 1988), we deduce from our results that a rain shower -- as emulated by the single acid treatments of our study -- would have to be strongly acidic (pH < 3) to injure Douglas-fir seedlings. Lodgepole pine seedlings are apparently even more insensitive to direct acid effects on their foliage. Although pretreatment chlorosis may have reduced our capacity to identify acid effects on the lodgepole pine, we conclude that our experimental design and factorial analysis of variance was adequate for recognizing differences due to the acid treatments

-- and that in fact, these species are not likely to be injured by a single acid event, at acidity below pH 2. This conclusion is consistent with the finding that white pine (Pinus strobus) needle injury appeared only when acidity increased to pH 1 and stronger (Haines and others 1980). We therefore conclude that a single acid event at concentrations less than pH 2 will have little lasting effect on lodgepole pine and Douglas-fir seedlings. Older forest-grown trees are probably even less susceptible to injury by occasional acid rain events because their needles have thicker cuticles (which may restrict penetration by pollutants) and are less wettable (due to lower contact angles), than are seedlings --especially those grown in a greenhouse (Schreiner 1986).

Because the literature shows that repeated acid applications are injurious, one must still be concerned about recurrent as well as intense acid events. For example, ponderosa pine (Pinus ponderosa), Douglas fir, and white spruce (Picea glauca) seedlings were seriously injured by seven to twelve acid applications at pH 2 (McColl and Johnson 1983, Abouguendia and Baschak 1986). Also, thresholds of acid sensitivity may vary significantly among species; for example, white spruce was judged sensitive and jack pine (Pinus banksiana), a close relative of lodgepole pine (Mirov 1956), was judged to be very resistant (Abouguendia and Baschak 1986). Hence, after multiple exposures, thresholds of acid sensitivity may vary significantly among species (Percy 1986). Not all acid events are necessarily

detrimental; however. Some beneficial effects of low intensity acid events are found in the literature. In short-term experiments, repeated applications of dilute H_2SO_4 or HNO_3 solutions (pH 2.3-5.6) were found to sometimes stimulate growth of pines, junipers, and Douglas-fir -- suggesting sulfur and nitrogen fertilization (Lee and Weber 1979 and Wood and Borman 1971). Similarly, increased photosynthesis has been reported for loblolly pine (Pinus taeda Wong et al 1990) and red spruce (Kohut et al 1990) needles exposed to pH 3 acid rain.

Metal Effects

Metals are absorbed by the roots of plants (Bazzaz et al 1974, Carlson et al 1975, Chino 1981, Schlegel 1987) and reduce rates of respiration, transpiration, photosynthesis, and growth when the metal levels are too high (Van Assche and Clijsters 1983, Schlegel 1987). Electron micrography suggests that chloroplasts are the organelles most sensitive to injury from metals (Guderian 1986); thus, it seems reasonable to assume that the observed foliage injury from metals in our study was accompanied by reductions in at least some of the above-mentioned physiological processes -- and that this especially might increase with repeated foliar applications of the metals.

From the significant increases in needle injury we found with each metal chloride, we conclude that foliar exposure of conifer seedlings to such metals can be injurious. In fact, this

conclusion appears to hold equally for lodgepole pine and Douglas-fir, except that $PbCl_2$ at 10^{-1} M concentration significantly affected Douglas-fir but did not significantly affect lodgepole pine. However,one expects toxicity at different metal concentrations, for different metals, because Hg, Cd, and Pb are generally more toxic to protoplasm than Cu and Zn (Chino 1981,Taylor and others,1990) . The common concentration (10^{-2} M) generally observed for the onset of statistically significant metal chloride toxicity in our study (Table 5) suggests that some barrier -- likely the cuticle -- is finally saturated at this concentration and on saturation allows absorption of metals which poison the cells within. If so, the surprisingly low needle injury we observed from Pb must be due to a superior capability of this relatively insoluble metal chloride to bind to the cuticles of conifer needles -- especially with lodgepole pine. Conversely, the significant injury to foliage from Cu, although less toxic to plant tissue than Pb, is apparently due to its relatively greater penetration of needle cuticles.

While the metal concentrations which had foliar effects in our study are generally higher than the 10^{-3} to 10^{-4} M concentrations found in precipitation of highly polluted urban-industrial areas (Jeffries and Snyder 1981), concentrations within trees might reach levels sufficient to produce foliage injury comparable to that produced by our single exposures, after 10-100 rain showers -- if the material accumulated in the trees. Metals from air-borne pollution build up in the soil and become mobilized by

soil acid as it accumulates from persistent pollution (Smith 1991). Acid-mobilized metals are absorbed by roots of Rocky Mountain conifers and accumulate to injurious levels, as seen in the metal-sulfide poisoning of conifers near smelters at Anaconda, Montana and Kellog, Idaho. From our results, therefore, we find it reasonable to accept the conclusion that while single acid and metal air pollution events at high concentrations can injure seedling foliage of Rocky Mountain conifers, root-absorbed metals are the primary toxic component in the poisoning of conifer forests near smelters -- and acid pollution facilitates the process.

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Table 1. Concentrations of metal chloride solutions in parts per million (ppm).

Metal Chloride	mw[1]	10^{-1}M	10^{-2}M	10^{-3}M	10^{-4}M
$CdCl_2$	183.4	18340	1834	183.4	18.3
$CuCL_2 * 2H_2O$	134.5	13450	1345	134.5	13.4
$HgCl_2$	271.5	27150	2715	271.5	27.2
$PbCl_2$	278.0	27800	2780	278.0	27.8
$ZnCl_2$	136.4	13640	1364	136.4	13.6

[1]Mol wt. = gm/mole when diluted to 1 liter gives a 1 molar solution.

Table 2. Nominal effect of acids at different concentrations vs. distilled water controls, on lodgepole pine and Douglas-fir seedlings. Tabled values are mean percentages of injured (chlorotic and dead) needles.

Acid	Treatment				
	Control	pH5	pH4	pH3	pH2
			Lodgepole Pine		
HCL	15.1	17.9	13.9	18.1	17.3
H_2SO_4	20.5	14.9	18.8	18.5	29.2
HNO_3	15.5	12.6	20.6	14.8	14.3
			Douglas-fir		
HCL	2.0	2.7	4.0	1.0	0.0
H_2SO_4	0.5	0.9	0.0	1.6	16.4
HNO_3	0.2	0.0	0.0	0.3	6.9

Table 3. Nominal effect of metal chlorides at different concentrations vs. distilled water controls, on lodgepole pine and Douglas-fir seedlings. Table values are mean percentages of injured (chlorotic and dead) needles.

Metal Chloride	Treatment				
	Control	10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M
			Lodgepole Pine		
$HgCl_2$	22.0	21.5	17.3	49.7	95.8
$CdCl_2$	15.8	13.7	20.7	54.7	90.0
$CuCl_2$	26.4	14.0	29.1	72.2	45.2
$ZnCl_2$	15.9	16.9	16.8	34.7	74.3
$PbCl_2$	11.2	6.9	27.8	14.0	17.6
			Douglas-fir		
$HgCl_2$	6.9	0.8	0.8	20.7	82.6
$CdCl_2$	0.0	0.7	15.7	4.8	13.0
$CuCl_2$	2.0	0.2	5.7	11.1	16.9
$ZnCl_2$	1.1	3.4	1.1	4.9	45.6
$PbCl_2$	1.1	2.6	0.5	0.6	8.7

Table 4. P-values of differences in least squares means of needle injury, between distilled water controls and acid treatments, on lodgepole pine and Douglas-fir seedlings. Analysis of variance was based on ranked values of percentage of needles showing injury.[1]

Acid	Treatment Concentration			
	pH5	pH4	pH3	pH2
		Lodgepole Pine		
HCL	0.5048	0.7244	0.6048	0.9503
H_2SO_4	0.2336	0.6006	0.6925	0.4841
HNO_3	0.3701	0.7372	0.5614	0.5350
		Douglas-fir		
HCL	0.5059	0.2447	0.6812	0.4741
H_2SO_4	0.4167	0.2539	0.5865	**0.0004**
HNO_3	0.4438	0.4438	0.9385	**0.0026**

[1] Ranking was required to satisfy normality assumptions in the analysis.

Table 5. P-values of differences in least squares means of needle injury, between distilled water controls and metal chloride treatments, on lodgepole pine and Douglas-fir seedlings. Analysis of variance was based on ranked values of percentage of needles showing injury.[1]

Metal Chloride	Treatment Concentration 10^{-4} M	10^{-3} M	10^{-2} M	10^{-1} M
		Lodgepole Pine		
$HgCl_2$	0.5715	0.8314	**0.0030**	**0.0001**
$CdCl_2$	0.3739	0.4519	**0.0004**	**0.0001**
$CuCl_2$	0.1784	0.6562	**0.0001**	**0.0023**
$ZnCl_2$	0.8431	0.7327	**0.0041**	**0.0001**
$PbCl_2$	0.3791	0.1152	0.7449	0.4878
		Douglas-fir		
$HgCl_2$	0.9145	0.8171	**0.0088**	**0.0001**
$CdCl_2$	0.4012	0.0457	**0.0230**	**0.0020**
$CuCl_2$	0.2572	0.1262	0.0157	**0.0030**
$ZnCl_2$	0.7259	0.9440	0.0100	**0.0001**
$PbCl_2$	0.5352	0.8153	0.9268	**0.0395**

[1] Ranking was required to satisfy normality assumptions in the analysis.